

## DYNAMICS OF OVARIAN FOLLICLES IN BREEDING DUCKS

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ABSTRACT.—I quantified ovarian rapid follicle growth (RFG) and regression of postovulatory follicles of Northern Pintails (*Anas acuta*), American Wigeon (*A. americana*), and Lesser Scaup (*Aythya affinis*) by a method that accounted for within-day variation in follicle size. Objective methods for identifying onset of RFG also are presented; this is crucial for accurate classification of breeding status. Duration of RFG was estimated as 4.2, 5.1, and 5.0 days for pintails, wigeon, and scaup, respectively; these are shorter than previously reported. Diameters of follicles at the beginning of RFG were estimated to be 8.2, 6.9, and 7.9 mm for pintails, wigeon, and scaup, respectively. For all species, RFG was linear, using follicle diameters, and exponential, using dry masses. Models of RFG and postovulatory follicle regression have practical value for calculating nest initiation dates, number of developing follicles, clutch size, reneating intervals, and daily energy and nutrient commitment to reproduction of collected breeding females. Received 12 November 1993, accepted 20 April 1994.

Rapid follicle growth (RFG) is the period from the time an ovarian follicle begins rapidly accumulating yolk until ovulation (see Lofts and Murton [1973] for descriptions of ovary structure and control). In ovaries of breeding birds, initiation of RFG of successive follicles is staggered in accordance with egg-laying interval. As a result, developing follicles have a distinct size hierarchy that corresponds to the order in which they will be ovulated. Postovulatory follicles are the follicle structures remaining after ovulation (Lofts and Murton 1973); they regress over time, resulting, similarly, in a size hierarchy within an ovary. Based on this information and assumptions about rates of egg laying, models of RFG and postovulatory regression through time can be developed.

Previous studies have described ovarian follicle growth based on changes in mean follicle size by day (e.g., Calverley and Boag 1977, Astheimer and Grau 1990, Alisaukas and Ankney 1992) but did not present model equations. No previous investigators presented continuous models of RFG or postovulatory follicle regression with predictive capabilities that could be used in subsequent studies.

My objective was to quantify ovarian follicle dynamics of Northern Pintails (*Anas acuta*; hereafter pintails), American Wigeon (*A. americana*; hereafter wigeon), and Lesser Scaup (*Aythya affinis*; hereafter scaup) by methods that accounted for within-day variation and objectively identified

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onset of RFG. I also present models that can be used to discern aspects of breeding biology from ovaries of collected females.

#### METHODS

Female pintails were collected in 1990 and 1991 at study sites on Yukon Delta National Wildlife Refuge (NWR) (61°26'N, 165°27'W) and Yukon Flats NWR (66°25'N, 149°59'W), Alaska. In 1991, female wigeon and scaup were collected on Yukon Flats NWR. Ovaries were removed and preserved in 10% formalin. In the laboratory, largest diameters in the plane of the stigma of preovulatory and postovulatory follicles were measured. Dry masses of preovulatory follicles were recorded. Because follicles preserved in formalin may be fixed in deformed shapes, dry masses of preovulatory follicles may be more accurate than diameters. However, analyses using diameters are advantageous because these measures are obtainable in the field or lab without additional processing.

Only laying females (i.e., those that had ovulated at least one follicle) were used as samples for modeling, because only those could have a general hierarchy assigned by day. Ovaries with follicles broken during collection or dissection were included only if the position in the hierarchy of the broken follicle was known with certainty. For late-layers with a gap in the follicle hierarchy, only large, developing follicles were used.

Within each ovary, follicles were assigned to a DAY, which was a rough estimate of the time before ovulation. For example, the largest follicle from each ovary was assigned DAY = 1, and it was assumed that it would have ovulated with 24 h. The second largest follicle was assigned DAY = 2, and so forth. Sample sizes by species and DAY are presented in Table 1. For these analyses, I assumed constant laying intervals of 24 h for all species (Alisauskas and Ankney 1992).

Rather than describe a rough growth curve based on mean follicle size for each DAY, I incorporated within-day variation in follicle sizes into continuous models. I corrected DAY (CORRDAY) for individual birds, using an adjustment based on that bird's largest follicle dry mass (DRY) relative to the range in mass between the smallest DAY 1 follicle (SMLFOLL) of the species (Table 1) and an estimate of the individual's follicle mass at ovulation (LRGFOLL). LRGFOLL was either (1) dry mass of the individual's oviductal egg yolk or (2) average yolk dry mass from a sample of oviductal and laid eggs. The former was used, when possible, to account for variation in egg composition among individuals, which is greater than variation within clutches (Duncan 1987). Thus, CORRDAY estimated time before ovulation for the largest follicle of each individual as:  $CORRDAY = (LRGFOLL - DRY)/(LRGFOLL - SMLFOLL)$ . For other follicles of each individual, CORRDAY was calculated by adding DAY for each follicle and CORRDAY from the largest follicle.

CORRDAY for the largest postovulatory follicle of each ovary (i.e., days after ovulation) was estimated using the correction for developing follicles:  $CORRDAY = 1 - (LRGFOLL - DRY)/(LRGFOLL - SMLFOLL)$ . Because postovulatory follicle diameters were subject to more measurement error, CORRDAY based on preovulatory follicles likely was more accurate than deriving a correction factor based on postovulatory follicle sizes.

I used an iterative approach to quantify beginning of RFG for each species. First, I used linear regressions to describe relationships between CORRDAY and follicle diameter for data sets consisting of (1) follicles clearly before RFG (i.e.,  $CORRDAY \geq 6.0$ ) and (2) follicles definitely in RFG (i.e.,  $CORRDAY < 3.5$ ). Exclusion of that range of points avoided using data near the beginning of RFG for all species (Fig. 1). In the second iteration, separate linear regressions were used to describe data less and greater than CORRDAY at the intersection of models from the first iteration. The intersection of models from the second

TABLE 1  
OVARIAN FOLLICLE SIZES OF BREEDING DUCKS

Day <sup>a</sup>		Northern Pintail		American Wigeon		Lesser Scaup	
		Diameter (mm)	Dry mass (g)	Diameter (mm)	Dry mass (g)	Diameter (mm)	Dry mass (g)
1	Range	26.3–33.4	4.56–7.80	28.3–34.5	5.63–8.18	30.8–35.6	7.27–10.63
	Mean	29.6	6.32	31.4	6.94	33.4	8.71
	N	42	40	11	11	15	15
2	Range	20.1–28.4	1.94–5.37	22.7–28.5	2.95–5.66	26.7–30.8	4.12–6.19
	Mean	24.1	3.43	26.2	4.27	28.8	5.27
	N	42	42	10	10	14	14
3	Range	14.4–23.2	0.65–2.62	17.8–23.3	1.30–2.75	18.6–24.7	1.50–3.93
	Mean	17.8	1.38	20.5	2.03	22.0	2.55
	N	41	41	8	8	14	14
4	Range	9.3–16.3	0.13–1.06	11.0–18.9	0.27–1.43	11.4–18.5	0.25–1.24
	Mean	12.0	0.42	14.6	0.78	15.5	0.81
	N	34	31	9	8	15	15
5	Range	6.0–11.8	0.01–0.34	7.6–14.4	0.07–0.57	8.2–13.2	0.06–0.40
	Mean	8.1	0.10	10.0	0.24	10.5	0.20
	N	26	21	7	6	13	12
6	Range	5.3–8.2	0.01–0.09	5.9–10.9	0.02–0.22	6.8–8.9	0.02–0.07
	Mean	6.8	0.04	7.3	0.08	7.7	0.05
	N	26	18	9	6	11	8
7	Range	4.7–7.5	0.01–0.05	6.1–7.2		6.4–8.1	0.03–0.05
	Mean	6.2	0.02	6.7		7.2	0.04
	N	24	12	7		10	5

<sup>a</sup> Where the largest in a series of developing follicles from laying females (which would have ovulated within 24 hours) = Day 1, the next largest = Day 2, etc.

iteration estimated CORRDAY and follicle diameter at the onset of RFG. I calculated 95% confidence limits around the CORRDAY estimate (Sokal and Rohlf 1981:498).

Polynomial models of RFG (i.e., for data with CORRDAY less than the estimate of beginning of RFG) and postovulatory follicle regression were created to describe relationships between follicle sizes and CORRDAY, with CORRDAY up to the third order. Higher-order variables were removed if nonsignificant ( $P > 0.01$ ). Polynomial models also were derived with CORRDAY as the dependent variable, so that CORRDAY could be predicted from follicles of collected birds.

## RESULTS

CORRDAY (and 95% confidence limits) at the beginning of RFG (i.e., duration of RFG) were estimated to be 4.2 (3.8–4.6), 5.1 (4.7–5.6), and 5.0 (4.5–5.4) days for pintails, wigeon, and scaup, respectively; follicle diameters were estimated as 8.2, 6.9, and 7.9 mm, respectively.

Follicle diameters were linearly related to CORRDAY during RFG for all species (Fig. 1). Model intercepts estimated diameters at ovulation as 32.9, 33.8, and 37.1 mm for pintails, wigeon, and scaup, respectively.

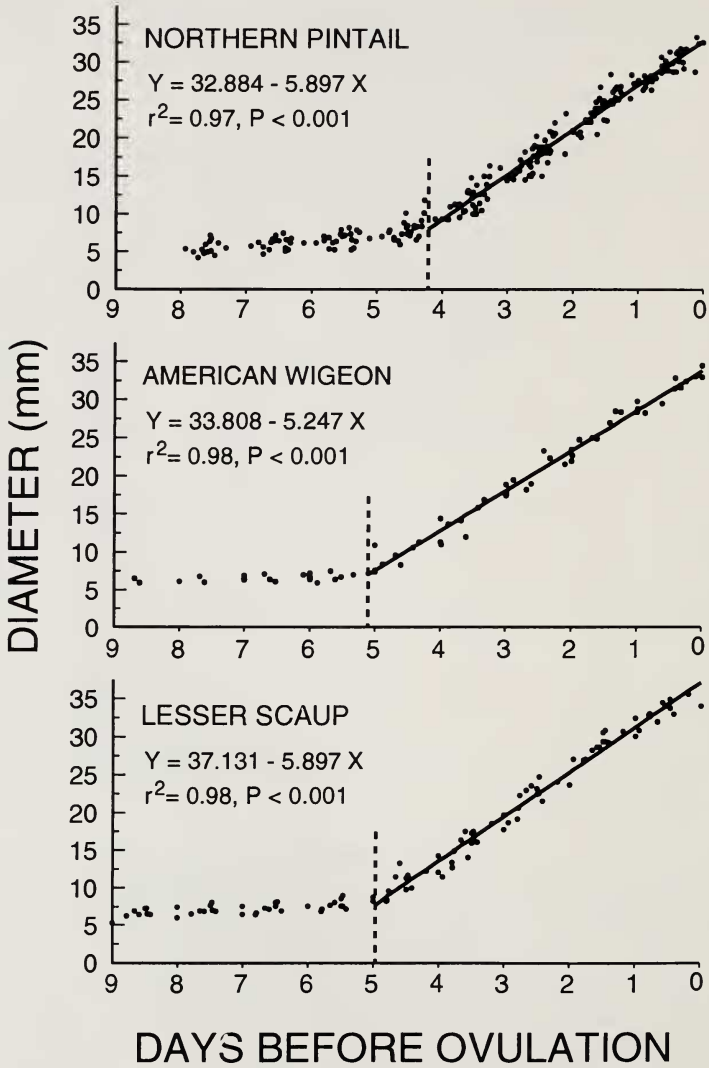


FIG. 1. Rapid follicle growth of three duck species based on ovarian follicle diameters. Vertical dashed lines represent estimates of beginning of rapid follicle growth.

Growth curves of follicle dry masses were best fit with second-order polynomial expressions (Fig. 2). Follicle dry masses at ovulation were estimated to be 8.2, 8.3, and 11.0 g for pintails, wigeon, and scaup, respectively. Predictive models of RFG (Table 2) estimated CORRDAY

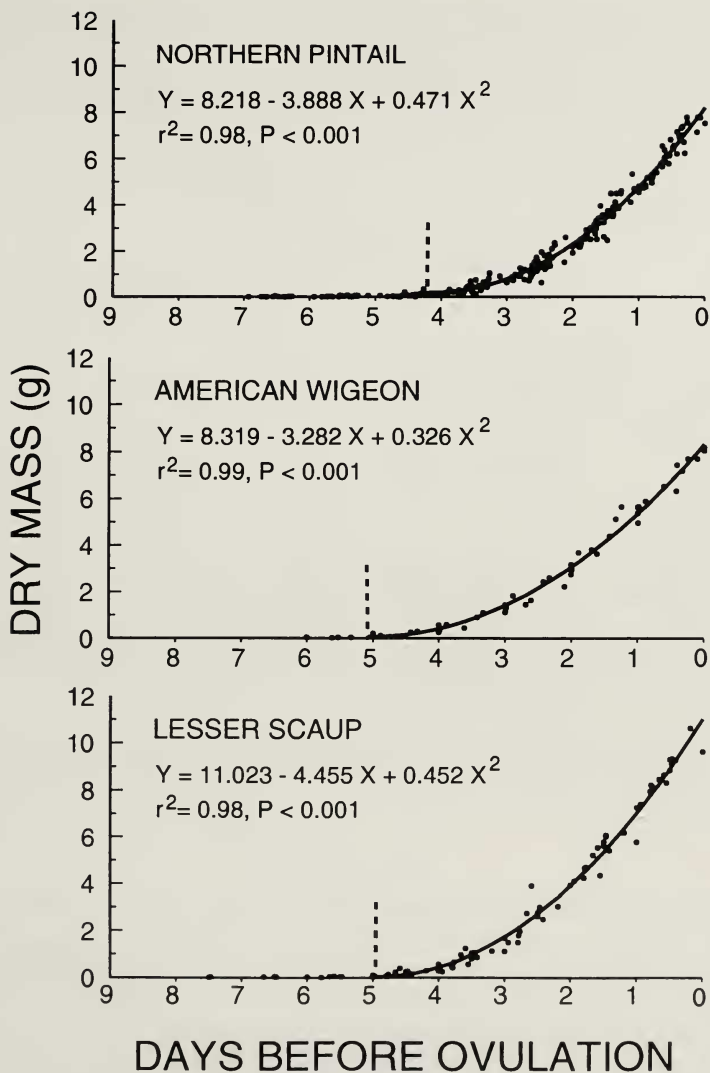


FIG. 2. Rapid follicle growth of three duck species based on ovarian follicle dry masses. Vertical dashed lines represent estimates of beginning of rapid follicle growth.

with linear models of diameter and third-order polynomials of dry mass for all species.

Postovulatory follicle regression was described by second-order polynomials for all species (Fig. 3). Intercepts of these models estimated post-ovulatory follicle diameter immediately after ovulation as 15.8 mm for

TABLE 2  
 PREDICTIVE MODELS<sup>a</sup> ESTIMATING CORRDAY<sup>b</sup> FROM OVARIAN FOLLICLE DIAMETERS (DIA)  
 AND DRY MASSES (DRY)

Group	Equation
Rapidly growing follicles	
Northern Pintail	
Diameter	$\text{CORRDAY} = 5.451 - 0.164\text{DIA}$
Dry mass	$\text{CORRDAY} = 3.894 - 1.162\text{DRY} + 0.168\text{DRY}^2 - 0.011\text{DRY}^3$
American Wigeon	
Diameter	$\text{CORRDAY} = 6.377 - 0.187\text{DIA}$
Dry mass	$\text{CORRDAY} = 4.800 - 1.607\text{DRY} + 0.263\text{DRY}^2 - 0.017\text{DRY}^3$
Lesser Scaup	
Diameter	$\text{CORRDAY} = 6.217 - 0.166\text{DIA}$
Dry mass	$\text{CORRDAY} = 4.524 - 1.088\text{DRY} + 0.134\text{DRY}^2 - 0.007\text{DRY}^3$
Postovulatory follicles	
Northern Pintail	$\text{CORRDAY} = 6.443 - 0.748\text{DIA} + 0.023\text{DIA}^2$
American Wigeon	$\text{CORRDAY} = 8.714 - 1.105\text{DIA} + 0.038\text{DIA}^2$
Lesser Scaup	$\text{CORRDAY} = 7.744 - 0.823\text{DIA} + 0.023\text{DIA}^2$

<sup>a</sup> All models  $r^2 > 0.96$ ,  $P < 0.001$  for rapidly growing follicles,  $r^2 > 0.87$ ,  $P < 0.001$  for postovulatory follicles.

<sup>b</sup> The number of days until ovulation for rapidly growing follicles and days since ovulation for postovulatory follicles.

both pintails and wigeon, and 18.2 mm for scaup. Predictive models (Table 2) can be used to estimate CORRDAY based on diameters of postovulatory follicles.

#### DISCUSSION

Consistent and objective criteria have not been used for defining beginning of RFG for ducks (i.e., defining a measure for distinguishing between developing and nondeveloping follicles), which is essential for determining breeding status. Ovary masses of 3.0 g have been used for pintails (Krapu 1974), Mallards (*Anas platyrhynchos*; Krapu 1981), and Ring-necked Ducks (*Aythya collaris*; Hohman 1986). Follicle diameters have been used for Ruddy Ducks (*Oxyura jamaicensis*; 8.0 mm; Tome 1984), Canvasbacks (*A. valisineria*; 7.5 mm; Barzen and Serie 1990), and pintails (6.0 mm; Phillips and van Tienhoven 1962, Mann and Sedingler 1993). Follicle dry mass of 0.10 g was used for Northern Shovelers (*Anas clypeata*; Ankney and Afton 1988) and Ring-necked Ducks (Alisauskas et al. 1990). Conservative estimates were obtained by using dry mass of the second smallest "developing" follicle from samples of hens with complete sets of follicles; criteria by this method have included 0.20 g for scaup (Afton and Ankney 1991), 0.40 g for Gadwall (*A. strepera*;

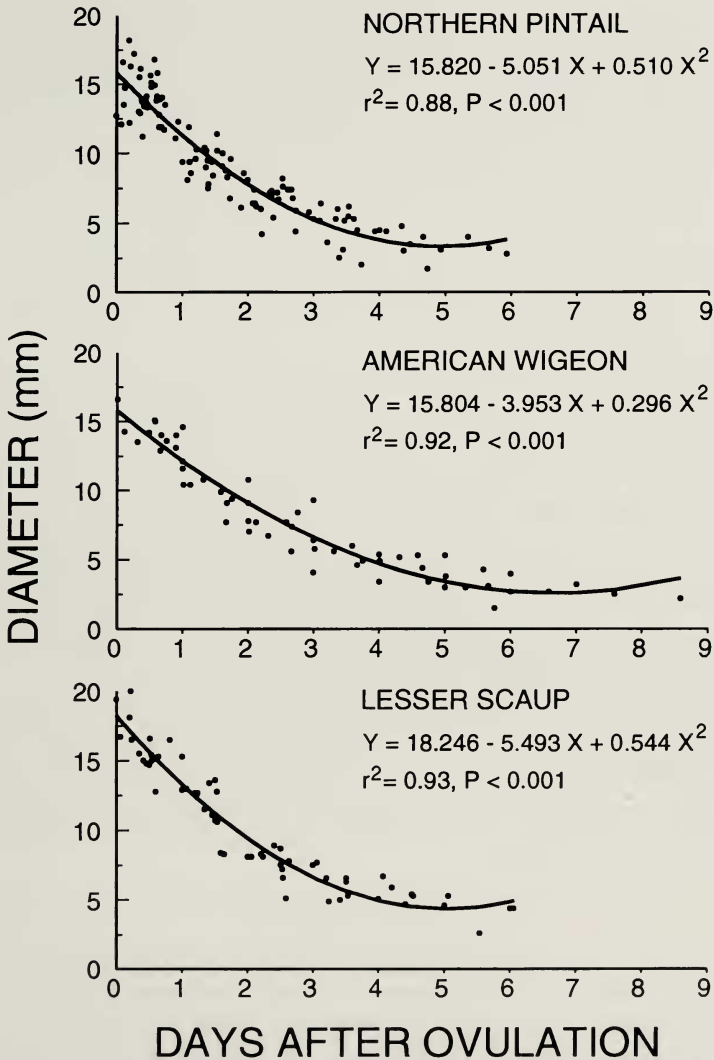


FIG. 3. Regression of postovulatory follicles of three duck species.

Ankney and Alisuskas 1991), and 0.39 g for Mallards (Young 1993). Clearly, it would be valuable to derive consistent methods for interpretation of breeding status from ovaries. Otherwise, there is danger of misinterpreting breeding status and affecting associated analyses.

Initiation of RFG can be determined objectively by the methods presented here. To apply this information to determine waterfowl breeding

status, I suggest adding a conservative buffer to follicle size estimates at the beginning of RFG to be certain that follicles are in RFG. For example, for the species in this study, 10 mm is an appropriate distinction between RFG and non-RFG follicles. Only three pintail follicles were >10 mm before the beginning of RFG (Fig. 1). From polynomial models describing relationships between follicle diameters and dry masses ( $P < 0.001$ ,  $r^2 > 0.98$ ), I found that 10 mm corresponded to 0.12, 0.15, and 0.10 g dry mass for pintails, wigeon, and scaup, respectively; thus, 0.15 g dry mass also is an appropriate distinction for these species. Follicles before the beginning of RFG were <0.15 g, again with the exception of the three pintail follicles.

Duration of RFG can be estimated in several ways. Rough estimates can be obtained by multiplying the maximum number of developing follicles by the egg-laying interval (Alisauskas and Ankney 1992). Renest intervals have been used as a maximum estimate (Grau 1984). Duration of RFG also has been estimated by examining rings in cross-sections of yolk that form as yolk material is deposited; each pair of rings was presumed to represent daily growth (e.g., Grau 1976, 1984; Roudybush et al. 1979; Astheimer and Grau 1990). However, Alisauskas and Ankney (1994) suggested that the ring method may not work for laying waterfowl with a diphasic feeding regime that may lay down more than one set of rings each day. This pattern was found in Japanese Quail (*Coturnix coturnix*) fed twice daily (Dobbs et al. 1976). The method I presented here has advantages over other methods because the results are more exact and, unlike the ring method, laboratory analyses are not required and assumptions regarding yolk deposition are not necessary. However, collection of birds is required.

My estimates of duration of RFG are shorter than the six days previously described for these species (Phillips and van Tienhoven 1962, Alisauskas and Ankney 1992). These results are corroborated by examining ranges and means of follicle sizes by DAY (Table 1) for each species; follicles were nondeveloped, on average, on DAY 5 (4–5 days from ovulation) for pintails and DAY 6 (5–6 days from ovulation) for wigeon and scaup. Without comparably treated data from mid-continent breeding areas, it is unknown if there is geographic variability in RFG duration. Short RFG duration may be advantageous for species that exploit unpredictable food resources or that experience high rates of nest predation (Alisauskas and Ankney 1992). However, shorter RFG results in increased daily costs of egg production (Alisauskas and Ankney 1992, 1994).

Although researchers have used ovary characteristics to determine waterfowl breeding status, other values of RFG models have not been exploited. When applied to ovaries of individuals, these models can identify



important aspects of their basic breeding biology. For example, nest initiation dates of birds with developing follicles can be estimated accurately by determining CORRDAY and adding a day for the time the follicle is in the oviduct (Alisauskas and Ankney 1992). Time of day of ovulation also can be estimated. Models of RFG allow detection of breaks in the follicle hierarchy of individuals late in their laying sequence, differentiating follicles that would be laid from those that would not; in such cases, clutch size is the number of developing follicles plus the number of post-ovulatory follicles. Some analyses of nutrient reserves require accurate distinction of the number of follicles remaining to be laid (e.g., Ankney and Alisauskas 1991, Esler and Grand 1994). Renesting intervals can be determined by estimating days since ovulation of the last follicle of the first nest (using postovulatory follicle models) and days until laying of the first egg of the reneest (using models of RFG). Furthermore, for assessments of nutrient and energy commitment to clutch formation (e.g., Drobney 1980, Astheimer 1986, Alisauskas and Ankney 1994), RFG models could provide accurate daily changes.

Postovulatory follicles have been used as objective measures of clutch size and incidence of brood parasitism (e.g., Kennedy et al. 1989). Persistence of postovulatory follicles is variable among taxa (see review in Semel and Sherman 1991). Postovulatory follicles of Wood Ducks (*Aix sponsa*) were detectable for <30 days after ovulation (Semel and Sherman 1991); I suspect this is true for species in this study also. Models for pintails, wigeon, and scaup described regression of postovulatory follicles for only a few days after ovulation; these have value for determining clutch size and, for birds early in incubation, how long they have been incubating.

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